Plant Physiol. (1982) 69, 1353–1355
0032-0889/82/69/1353/03/$00.50/0

Abscisic Acid Transport Coefficients of *Phaseolus* Root Systems[1]

Received for publication August 18, 1981 and in revised form December 17, 1981

EDWIN L. FISCUS, FRANK R. STERMITZ, AND LUIS G. AMOROS
United States Department of Agriculture-Agricultural Research Service, Crops Research Laboratory (E. L. F.), and Department of Chemistry (F. R. S., L. G. A.), Colorado State University, Fort Collins, Colorado 80523

ABSTRACT

Diffusive and convective transport coefficients of *Phaseolus vulgaris* L. cv. Ouray root systems for abscisic acid for (ABA) were measured. The convective coefficient (reflection coefficient or osmotic efficiency factor) σ was determined to be 0.96 for ABA while the diffusive coefficient, ω, was found to be 1.44×10^{-11} mole per square centimeter per second per bar. Steady-state concentrations of ABA in the root system exudates were not achieved until at least three hours after the applications suggesting either a slow saturation of binding sites or equilibration with tissues surrounding the xylem.

Much of the methodology for determining the mode of action of ABA in the whole plant or in organs depends upon a supply of exogenous ABA. ABA has been applied to plants or plant parts in a wide variety of ways. One especially useful mode of application, which allows the plant to remain intact, is adding the ABA to the root medium of hydroponically grown plants. A major problem with this technique is that there is usually no information available concerning the quantities of ABA which actually are transported to the sites of action or concerning the time course of such transport. This information is vital to the interpretation of the physiological responses of leaves to ABA applied to the roots. It is this particular point we will address in this paper by presenting the time course for ABA transport through *Phaseolus* root systems and calculating the diffusive and convective transport coefficients for ABA in these systems. Knowledge of these coefficients should greatly facilitate interpretation of those ABA experiments where the growth regulator is applied to the root medium.

MATERIALS AND METHODS

Experimental Procedures. Bean seeds (*Phaseolus vulgaris* L. cv. Ouray) were germinated in vermiculite for 4 d then transferred to 5-L plastic pots filled with aerated half-strength Hoagland solution and grown in a greenhouse. The pots were topped up daily with distilled H_2O and the solution changed completely once a week. Supplemental lighting gave a mean midday photosynthetic photon flux density of 425 μE m^{-2} s^{-1} at the top of the pot. The root systems were nonnodulated. When the plants were 2 to 4 wk old they were decapitated and sealed into a pressure chamber where the solute and volume fluxes were determined. The cut stump of the plant protruded through the lid of the chamber and the roots were surrounded by aerated nutrient solution as previously described (1, 2). Projected leaf areas were measured with a LI-COR LI3100[2] area meter. The chamber was brought to the specified pressure and temperture (25 ± 0.25°C) and equilibrated overnight. In the morning, the system was checked for steady-state conditions, arbitrarily specified as changes in the volume and solute fluxes of less than ± 2% h^{-1}. The total volume flux was measured at regular intervals and was expressed as the total volume flux per unit leaf area Q_{pl},[3] as a means of comparing plants of different sizes (2). Unpublished data from this laboratory show that the ratio of projected leaf area to root surface area is constant and approximately 1 so that Q_{pl} will approximate the volume and solute flux densities through the roots. The electrical conductivity of the exudates was measured and the concentration expressed as equivalents of KCl cm^{-3}. The samples were immediately frozen for future ABA analysis and for osmotic pressure determinations by freezing point depression. Having established steady conditions according to the above criteria, the ABA (Sigma ± cis-trans), dissolved in 95% ethanol, was added to the system through an injection port without decreasing the pressure. The quantity of ABA added was calculated to yield the same approximate dose (1.5×10^{-7} mol cm^{-2} leaf area) in each case.

ABA Analysis. Analyses of ABA in the exudates and nutrient solutions were conducted using HPLC. A μ-Porasil column was used in a Waters Associates system with a model 6000A pump operating at 3,000 p.s.i. The flow rate was 2.0 ml min^{-1} and a UV detector was used operating at 254 nm. The eluting solvent was prepared by shaking a mixture of 350 ml acetonitrile and 700 ml chloroform with 300 ml 0.17 N acetic acid. The organic layer was separated and run through a micropore filter prior to use. With these conditions and solvent, the ± cis-trans ABA had a retention time of 4.0 minutes.

One-ml samples of nutrient solution or exudate were brought to about pH 2 by the addition of 4 drops of 1 M HCl. The solutions were then extracted 3 times with 1.5 ml ethyl acetate. The ethyl acetate layer was evaporated to dryness *in vacuo* at 35°C. Acetonitrile (100 μl) was added to dissolve the residue and 10 μl (nutrient solution), or 50 μl (exudate) injected onto the HPLC column. The area under the peak for ABA was then compared with the area under the same peak resulting from injection of a standard ABA solution. The extraction gave 85 to 90% recovery, and reproducibility in a set of four independent trials on ABA-spiked nutrient solution was within 10%. Standard ABA was injected at intervals between samples when an analysis was being run.

A large peak with the identical retention time could be detected in the exudates when ABA was added to the root medium. Repetitive injections of the exudate extracts were made and the same peak collected. TLC analysis (silica gel, ethyl acetate-methanol, 95:5) gave an R_f 0.24 spot under iodine visualization which was identical with the spot from standard ABA. MS of the

[1] Supported in part by the Colorado Agricultural Experiment Station, Fort Collins, Colorado.

[2] Mention of companies or commercial products does not imply recommendation or endorsement by the United States Department of Agriculture over others not mentioned.

[3] Abbreviation: Q_{pl}, total volume flux per unit projected leaf area.

collected exudate peaks showed the presence of the three major ABA fragments at m/e 134, 149, and 162, although the m/e 264 molecular ion peak could not be distinguished from background peaks.

A pooled sample of 6 ml of exudate obtained before the addition of ABA was examined and found to show two very small peaks in the ABA region. Coinjection of standard ABA gave a peak which was close, but not identical with the two observed peaks from the pooled sample. Our method was thus unable to detect any endogenous ABA in xylem exudates.

A sample of mixed isomers (Sigma) was used to check for the possibility of isomerization of ABA in the nutrient solution or the plant. The sample was a mixture of cis-trans and trans-trans forms in a 1:2.5 ratio as shown by ^{1}H and ^{13}nmr analysis. The isomers were separable by HPLC with the trans-trans from having a retention time of 3.5 min a opposed to 4.0 min for the cis-trans form. Analysis of the nutrient solution and the exudates throughout the experiments showed only the presence of the cis-trans peak.

Transport Coefficients. Transport coefficients for ABA, water, and solutes were determined by the procedures of Fiscus (1) based on the transport equations

$$J_v = L_p\Delta P - \sigma RT(C^o - C^i), \quad \text{and} \tag{1}$$

$$J_s = C^o(1 - \sigma)J_v + \omega\Delta\Pi + J_s^* \tag{2}$$

J_v is the volume flux density in cm^3 cm^{-2} s^{-1}, L_p the hydraulic conductance in cm^3 cm^{-2} s^{-1} bar^{-1}, ΔP and $\Delta\Pi$ the hydrostatic and osmotic pressure differences across the root in bars, σ the osmotic efficiency or reflection coefficient, J_s the solute flux density in mole cm^{-2} s^{-1}, C the concentration in mole cm^{-3}, the superscripts o and i refer to the outside and inside of the root respectively, ω the coefficient of solute mobility in mole cm^{-2} s^{-1} bar^{-1}, R the universal gas constant in cm^3 bar mol^{-1} K^{-1}, T the absolute temperature in K, and J_s^* the active solute transport rate in mole cm^{-2} s^{-1}. Data from 16 plant systems ranging in size from 750 to 2,500 cm^2 projected leaf area were combined and analyzed as one system.

RESULTS AND DISCUSSION

Pre-ABA steady state volume fluxes and exudate concentrations are shown in Figure 1 as a function of applied pressure (ΔP). The

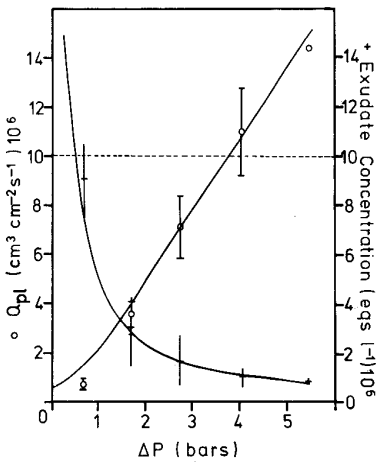

FIG. 1. Combined volume flux and exudate concentrations for the sixteen plants used. Points are means for each pressure and the bars are standard deviations. The number of plants, n, for each level of ΔP are 2, 5, 3, 5, and 1 going from low to high ΔP. Solid lines are theoretical curves calculated from equations 1 and 2 and the values in the text. The dashed horizontal line is the concentration of the nutrient solution.

Table I. *Calculated Values of Transport Coefficients*

Pre-ABA values are for all the components of the nutrient solution. ABA values are for ABA only.

	σ	ω	L_p
		mol cm^{-2} s^{-1} bar^{-1}	*cm^3 cm^{-2} s^{-1} bar^{-1}*
Pre-ABA	1.00	1.19×10^{-11} [a]	2.90×10^{-6}
ABA	0.96	1.44×10^{-11}	

[a] The figure given is actually $\omega\Pi^o + J_s^*$ mol cm^{-2} s^{-1}.

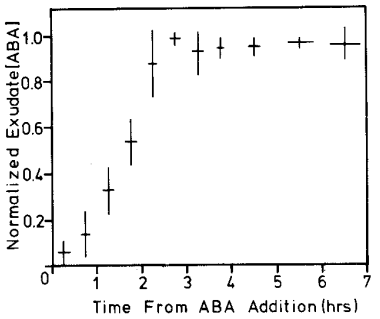

FIG. 2. Normalized ABA concentration in root exudate. Vertical and horizontal lines are ± 1 SD in concentration and time. Progressing through time, n for each point is 7, 9, 10, 7, 5, 5, 6, 3, 8, 3, and 4.

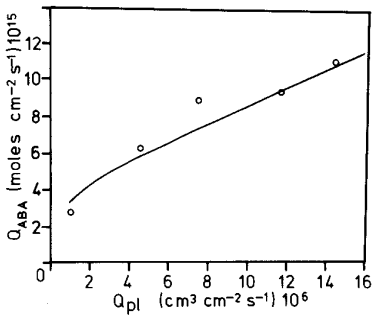

FIG. 3. ABA transport out of the root systems as a function of volume flux. The circles are the means of the data for each level of ΔP and the solid line is calculated from equation 2 and values given in Table 1.

large standard deviations in Figure 1 are probably due to the variation in conductance of the root systems with plant size (2) and we used a fairly wide range of plant sizes in this study. However, the analysis was performed on the means for each set with the result that the combined σ for all the components of the nutrient could not be shown to differ from 1.00 and L_p was calculated as 2.9×10^{-6} cm^3 cm^{-2} s^{-1} bar^{-1} (Table I). For reasons previously discussed (1) ω and J_s^* could not be separated by this technique so a combined figure for $\omega\Pi^o + J_s^* = 1.19 \times 10^{-11}$ mol cm^{-2} s^{-1} was calculated. Since $\sigma = 1$ we may speculate that ω is nearly zero and that the last number given reflects primarily the combined J_s^* for all the ionic species in the nutrient. If this number seems high, it is because it represents a summation for all the nutrients in the solution. Determination of these coefficients based on the freezing point depressions of the exudates yielded the same results so that the use of conductivity as a measure of the osmotic concentration appears to be justified in this instance. The coefficients presented thus far serve to demonstrate that the systems were behaving well and were comparable to those previously described (1, 2).

The buildup of ABA to steady concentrations in the xylem exudate is shown in Figure 2. Note that the concentration is expressed as a fraction of the maximum for any system inasmuch

as the actual concentrations varied widely. This variation was due to the fact that the exudate concentration of any transportable substance varies inversely with the volume flux rate. Also, since a period of 3 h was necessary to achieve steady-state concentrations, only values obtained after 3 h were used for the estimation of σ_{ABA} and ω_{ABA}. The values of volume flux density associated with steady ABA xylem exudate concentrations at various levels of ΔP were used to calculate σ_{ABA} and ω_{ABA} by the same method (1) that σ and ω were calculated for the pre-ABA nutrient solution except in this case only the transport of ABA was considered. Also, because there is probably no specific active transport mechanism for ABA, J_s^* may be deleted from equation 2. The result is that $\sigma_{ABA} = 0.96$ and $\omega_{ABA} = 1.44 \times 10^{-11}$ mol cm^{-2} s^{-1} bar^{-1}. This high value for ω_{ABA} is likely related to the solubility properties of ABA in biological membranes. Inserting these values into equation 2 allows calculation of the theoretical ABA flux density curve shown in Figure 3 along with the average measured values of J_{ABA} and Q_{pl} at the five levels of ΔP used (Fig. 1). Equation 2 seems to provide a reasonable fit to the data so it should be possible to predict the flux of exogenous ABA to the leaves from the root medium. The fit of Figure 3 also is consistent with the assumption that there is no active transport of ABA. However, if in future this assumption is proven false then the value for σ_{ABA} will have to be interpreted by equation 2 in terms of $\omega_{ABA}\Pi^o + J^*_{ABA}$ (1).

The slowness of approach to the steady concentration of ABA in the xylem is cause for caution in the use of our calculated transport coefficients. According to unpublished dimensional data from similar root systems, it seems that during the approximately 3 h until steady exudate concentrations occurred (Fig. 2), a quantity of exudate equal on average to about 10 times the total root volume was expressed. This relative volume is similar to what Markhart (4) observed in soybean. The causes for the shape of the time course are related to the transient changes in total volume fluxes described earlier (3). The additional possibilities exist that part of the lag may be due to slowness in saturating any specific ABA binding sites or in equilibrating with tissues around the xylem.

It is also important to realize that the calculations we have made reflect an average situation for the entire root system and that the mechanistic interpretation of ω and σ in these systems remains obscure. What may appear as exclusion of some solutes (high σ) may only reflect steady rates of immobilization or degradation. If we are dealing with active sites or binding sites, along the pathway of solution movement, it is clear that these sites must be partially satisfied before quantities of ABA will effectively pass through the roots and on to the shoot. Regardless of the actual mechanisms involved Figure 3 shows that equation 2 and the coefficients presented here may be used to estimate the transport of ± cis-trans ABA to the shoot. For example from equation 2 or Figure 3, at the dosages used here, and for a transpiration rate (Q_{pl}) of about 5 μg cm^{-2}s^{-1}, our figures predict a rate of ABA transport to the shoot of about 6×10^{-15} mol cm^{-2} s^{-1}. At the steady-state, therefore, we should see about 22 pmol ABA transported to each cm^2 of leaf area every h.

LITERATURE CITED

1. Fiscus EL 1977 Determination of hydraulic and osmotic properties of soybean root systems. Plant Physiol 59: 1013–1020
2. Fiscus EL 1979 Relationships between root system water transport properties and plant size in *Phaseolus*. Plant Physiol 64: 770–773
3. Fiscus EL 1981 Effects of abscisic acid on the hydraulic conductance of and the total ion transport through *Phaseolus* root systems. Plant Physiol 68: 169–174
4. Markhart AH 1982 Penetration of soybean root systems by abscisic acid isomers. Plant Physiol 69: 1350–1352

CORRECTION: Equation 1 should read:

$$J_v = L_p[\Delta P - \sigma RT(C^o - C^i)]$$